



Docket No.: CST-138 CIP2

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ABSTRACT

A method is provided for producing motif-specific, context-independent antibodies which recognize a plurality of peptides or proteins within a genome that contain the same motif. The method includes the step of immunizing a host with a degenerate peptide library antigen featuring (i) a fixed target motif containing one or more invariant amino acids including at least one modified amino acid, and (ii) a plurality of degenerate amino acids flanking the motif. Motif-specific, context-independent antibodies produced by the disclosed method are also provided. The method encompasses motifs consisting of a single modified amino acid, as well as short motifs comprising multiple invariant amino acids including one or more modified amino acids, such as all or part of kinase consensus substrate motifs, protein-protein binding motifs, or other cell signaling motifs. Methods of using the antibodies, e.g. for genome-wide profiling, are also provided.

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ABSTRACT

A method is provided for producing motif-specific, context-independent antibodies which recognize a plurality of peptides or proteins within a genome that contain the same motif. The method includes the steps of (1) constructing a degenerate peptide library featuring (i) a fixed target motif containing one or more invariant amino acids including at least one modified amino acid, and (ii) a plurality of degenerate amino acids flanking the motif; (2) immunizing a host with this peptide library to raise a context-independent antibody against all or part of the target motif, and (3) isolating antisera from the host and purifying the motif-specific, context-independent antibody from the antisera, the antibody recognizing a plurality of peptides or proteins within a genome that contain the motif. Motif-specific, context-independent antibodies produced by the disclosed method are also provided. The disclosed method encompasses motifs consisting of a single modified amino acid, [such as phosphothreonine, phosphoserine, phosphotyrosine, acetyl-lysine, and nitrotyrosine], as well as short [recurring] motifs comprising multiple invariant amino acids, [such as all or part of MAPK consensus substrate motifs, CDK consensus substrate motifs, PKA consensus substrate motifs, Akt consensus substrate motifs, PKC consensus substrate motifs, ATM consensus substrate motifs, 14-3-3 consensus binding motifs, PDK1 consensus docking motifs, phosphothreonine-X-(arginine(R)/lysine(K)), PKC Zeta consensus substrate motifs, ABL kinase consensus substrate motifs, CDK5 consensus substrate motifs, insulin receptor consensus substrate motifs, PI3K P85 consensus binding motifs, CaMKII consensus substrate motifs, SRC kinase consensus substrate motifs, CDC2/CDK2 consensus substrate motifs, GSK3 kinase consensus substrate motifs, and proline(P)-(phosphoserine/phosphothreonine)-proline(P)]. The antibodies of the invention are not limited, however, to these exemplary motifs. Methods of using the antibodies, e.g. for genome-wide profiling, are also provided.

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including
one or more
modified amino
acids, such as
all or part of
a kinase
consensus
substrate motifs,

protein-protein
binding motifs,
or other cell
signaling motifs.